

The biology of *Bracon celer* as a parasitoid of the olive fruit fly

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Abstract. A series of laboratory experiments was conducted on a colony of *Bracon celer* Szépligeti (Hymenoptera: Braconidae) reared on the olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). Female *B. celer* preferentially probe and oviposit into olives containing late third-instar fly larvae. The parasitoid develops as a solitary, ectoparasitic idiobiont. Mean development time (oviposition to adult eclosion) at 22 °C was, for females, 36 ± 1 (SE) days, and for males, 34 ± 1 days. The mean longevity of adult female wasps when provided honey and water was significantly greater than when they were provided water alone, or nothing. The females produced an average of 9.7 ± 7.2 progeny during their lifetimes, but production levels in the insectary colony suggested that this level of fecundity was artificially low and could be improved. The discrepancy may be a consequence of constraints on oviposition behavior imposed by the experimental design. The results are discussed with respect to insectary production methods and the potential use of *B. celer* as a biological control agent for olive fly in California.

Key words: *Bactrocera*, biological control, *Bracon*, Braconidae, Diptera, Hymenoptera, olive, parasitoid biology, Tephritidae

Introduction

Bracon celer Szépligeti (Hymenoptera: Braconidae) has been reported as the most abundant parasitoid attacking the olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in commercial and wild olives (*Olea* spp.) in the fly's native range in South Africa (Neuenschwander, 1982) and Kenya (Silvestri, 1914). Parasitism by *B. celer* at levels as high as 87% have been reported in South African olive orchards (Annecke and Moran, 1982). Because the olive fly has long been a major pest of olives in the Mediterranean basin (Clausen, 1978; White and Elson-Harris, 1992; Tzanakakis, 2003), *B. celer* has attracted interest as a potential biological control agent. Attempts to rear it have not been successful, however (Silvestri, 1914; Neuenschwander, 1982), and releases in Greece of adults shipped from South Africa did not result in establishment (Clausen, 1978; Wharton, 1989).

More recently, the olive fly arrived in California. It was discovered first in southern California in 1998 and it spread throughout the state within four years to pose a serious threat to the olive industry (Rice et al., 2003). Commercial growers apply insecticide bait to control it, but the effectiveness of these treatments is limited because infested trees in suburban and rural landscaping act as reservoirs for reinvasion into treated orchards. The costs and impracticality of treating non-commercial olive trees argue for the development of sustainable means of control. Furthermore, the effective biological controls that have been established for scale pests in California olives (Daane et al., 2005) may be disrupted by insecticides applied for olive fly. As in Europe, no effective natural enemies of olive fly exist in California, and thus a classical biological control program was initiated in 2002 (Hoelmer et al., 2004). Importation efforts have included foreign exploration for parasitoids of olive fly, as well as evaluation of parasitoids currently used to control other tephritids in the Mediterranean region and Hawaii (Sime et al., 2006).

Among the parasitoids reared from fly-infested olives collected in South Africa during the course of foreign exploration was *B. celer*. In June 2004, a small number was imported to California from South Africa via the USDA-ARS European Biological Control Laboratory (EBCL) in Montferrier, France, and used to establish a culture in the Insectary and Quarantine Facility at the University of California, Berkeley. To date, *B. celer* has been reported only as a parasitoid of olive fly and the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Tephritidae) (Wharton et al., 2000), with an additional, unconfirmed

record on *Ceratitis nigra* Graham (Narayanan and Chawla, 1962). Its host range may be larger, however, because it is thought to be an ectoparasitic idiobiont (Wharton et al., 2000), traits that are associated with relatively broad host ranges (Shaw, 1994). An unacceptable level of risk for parasitizing non-target species may preclude its release in California (Messing, 2000; Hoddle, 2004). On the other hand, its reported abundance on commercial and wild olives in its native range and its undeniably close association with olive fly indicate that *B. celer* merits investigation as a control agent. Because little is known about the biology of *B. celer*, and past attempts to have it reproduce in captivity have failed, we performed studies to improve rearing techniques and evaluate its potential as a biological control agent for the olive fly in California and elsewhere. We report herein on its biological characteristics and a rearing methodology, noting that its release in California is conditional upon completing assessment of non-target risks and comparing its performance with that of other imported parasitoids.

Materials and methods

Sources of insects and plants and colony maintenance

Laboratory cultures of olive fly were derived from infested olives collected near Davis, California (Yolo County). Flies were reared on olive fruit following the procedures of Tzanakakis (1989, 2003). Because the flies do not develop on small fruit less than 2 months old, and olives picked when fully ripe tend to rot before the fly larvae (or their parasitoids) complete development, we used a variety of olive cultivars (mostly Manzanillo, Sevillano, and Mission) with varying periods of ripening. These cultivars could be collected at different times across a wide area of the state (Riverside, Kern, Tulare, Fresno, and Yolo Counties), thereby providing fruit of an acceptable quality for 9–10 months out of the year. Olives held in cold storage were used for the remaining period. In general, olives were picked every 1–2 weeks and kept refrigerated until use.

Olives were exposed to adult flies in an oviposition chamber ($45 \times 45 \times 45$ cm³ wooden cage, with оргandy sides and a glass top) that was kept in a temperature-controlled insectary room (22 ± 2 °C, 16:8 L:D, 50% RH) at the Berkeley Insectary and Quarantine Facility. Adult flies had access *ad libitum* to water and a mixture (approximately 2:1 by volume) of honey and a dry yeast extract (FisherBiotech, Fairlawn, New Jersey, USA.). Olives were left in the cage for 1–2 days

or until they each had 5–10 oviposition marks. Infested olives were transferred to plastic boxes ($36 \times 18 \times 10 \text{ cm}^3$) with a nylon mesh top. To reduce mold growth, infested olives were placed in the box not more than 2–3 layers deep and were held about 2 cm off the bottom of the container by a metal grid. Under these conditions, the mature larvae exited the fruit and pupated on the bottom of the boxes after 10–14 days. Puparia were collected and transferred to the oviposition chamber to emerge as adults and repeat the process.

The adult wasps sent to California were obtained from fly-infested wild olives (*Olea europea* ssp. *cuspidata* (Wall ex G. Don)) collected in West Cape Province, South Africa, and in Oshikoto Province, Namibia, in April and May 2004. The olives were shipped by air to the EBCL in France, where the parasitoids emerged in quarantine. From this collection, 15 adult *B. celer* (8 females and 7 males) were identified and sent to the Berkeley Quarantine Facility in June 2004. There the wasps were placed in a $45 \times 45 \times 45 \text{ cm}^3$ cage that was freely provisioned with olive-fly infested olives, water, and a honey–water solution (50% by volume). Because *B. celer* was presumed an idiobiont, and its larvae had been observed feeding on third (last) instar olive fly larvae (Neuenschwander, 1982), we offered the parasitoids relatively mature fly larvae for oviposition. Olives infested 8–10 days earlier and thus containing a mixture of second and third instar hosts were exposed to parasitoids for 1–3 days, depending on parasitoid density. The inoculated material was then transferred to plastic rearing boxes as described above. The parasitoids reportedly spin cocoons within the feeding galleries of the fly larvae (Neuenschwander, 1982), and indeed all of the adult *B. celer* reared at EBCL from collections in 2002, 2003 and 2004 emerged directly from the fruit ($N=66$ in 2004), while none emerged from fly puparia that had dropped from fruit into holding containers. We therefore held the fruit for parasitoid emergence, recording the number of reared adults and condition of the fruit. Throughout the 6 months of colony maintenance, olive fruit were randomly selected from the colony and dissected to observe the condition and stage of parasitized hosts.

Host stages preferred for oviposition

Host-stage preference and oviposition success on different developmental stages of the olive fly were examined in choice tests. To produce an age series of olive flies, olives were exposed to adult flies for 8 h every 2 days and then held at $25 \pm 1^\circ \text{C}$. Immature stages

inside the olives were presented to the parasitoids when 2, 4, 6, 8, 10 and 12 days old. A sub-sample of olives from each set was dissected shortly before each test to determine which olive fly stages were present. Under these conditions, 2-day old olives contained eggs and, rarely, first instars; 4-day old olives contained first instars; 6-day old olives contained second instars; 8-day old olives contained second and young third instars; 10-day old olives contained third instars; and 12-day old olives contained mature third instars and were accompanied by prepupal larvae (emerging from fruit) and occasional pupae.

For each replicate, four female parasitoids were held for 24 h in an oviposition chamber (a plastic cylinder 13 cm deep \times 20 cm diameter, with a fine mesh top) with four olives of each of the six age classes. The olives were placed in the bottom of the container, grouped by age class in small plastic Petri dishes (5-cm diameter) marked with the age of the olives. There were 10 such replicates. During the first 7–8 h of the exposure period, ten brief (5-second) observations were made of the activity within the containers. The age class of the olives contacted by parasitoids was recorded. Also noted was whether the parasitoids were probing the olives with their ovipositors or simply standing on or off the olives but in the Petri dish.

Results are presented as means per age class (\pm SE), and treatment effects were analyzed using analysis of variance (ANOVA) with treatment means separated using Tukey's HSD test at $\alpha=0.05$. After the parasitoids were removed from the oviposition chamber, the olives were held at 25 ± 1 °C to rear either adult parasitoids or flies. Results are presented as means per age class (\pm SE), and treatment effects were analyzed using the Kruskal–Wallis test, the non-parametric analog of a one-way analysis of variance (SYSTAT 10.0, SPSS, 2000). To separate individual means, we used the Mann–Whitney test, the non-parametric analog of the two-sample *t*-test, for all possible pairwise comparisons of the treatment age classes, with an experiment-wide error rate at $\alpha' = \alpha/n = 0.0033$ ($\alpha=0.05$, $n=15$) (SYSTAT 10.0, SPSS, 2000).

Adult longevity and fecundity

Female longevity was compared among five treatments with access to (1) olives containing hosts, honey–water (50% by volume), and water; (2) uninfested olives, honey–water and water; (3) honey–water and water only; (4) water only; and (5) no provisions. Newly emerged females were collected daily, transferred to a small container with

males, supplied with water and honey–water, and held for 2 days to mate. The females were then randomly assigned to one of the five treatments, with each parasitoid isolated in a small plastic container (15 cm diameter \times 6 cm deep) with a hole (7 cm diameter) cut in the lid and covered with nylon mesh for ventilation. The olives (four per container) were replaced every other day. Where olives with hosts were offered, the fly larvae were at a suitable stage for parasitoid oviposition (mostly third instars). Each of the four olives bore 5–10 fly oviposition marks, and therefore 20–40 larvae were available for each 2-day interval. (This number was confirmed upon rearing, assuming that not all larvae survived to pupation: the mean number of puparia obtained in a subsample of 20 such replicates was 19.2 ± 2.2 .) Honey–water, streaked along the sides of the container, and distilled water, in a soaked cotton wick, were freely available. Parasitoids were checked daily for mortality. All treatments were kept in a temperature-controlled room (22 ± 2 °C, 50% RH, 16:8 L:D supplemented by natural daylight).

To determine lifetime reproductive potential, the infested olives that were collected every other day were held in plastic cups for emergence of adult flies or parasitoids. The number and sex of the emerging offspring were recorded.

Results are presented as means per treatment (\pm SE). Treatment effects were analyzed using proportional hazards modeling, a non-parametric survival analysis (SYSTAT 10.0, SPSS, 2000), with an experiment-wide error rate at $\alpha' = \alpha/n = 0.005$ ($\alpha = 0.05$, $n = 10$).

Pre-imaginal development time

The development time (oviposition to adult eclosion) of *B. celer* was assessed during insectary production. Infested olives were exposed to the parasitoid for about 24 h and then held at 22 ± 2 °C in plastic rearing containers. The containers were then checked every 1–2 days for adult fly or parasitoid emergence.

Results and discussion

Host stages preferred for oviposition

The parasitoids were most often observed searching on and probing in olives that had been infested 12 days earlier and contained mature third-instar hosts (Figure 1). Of the *B. celer* offspring that were

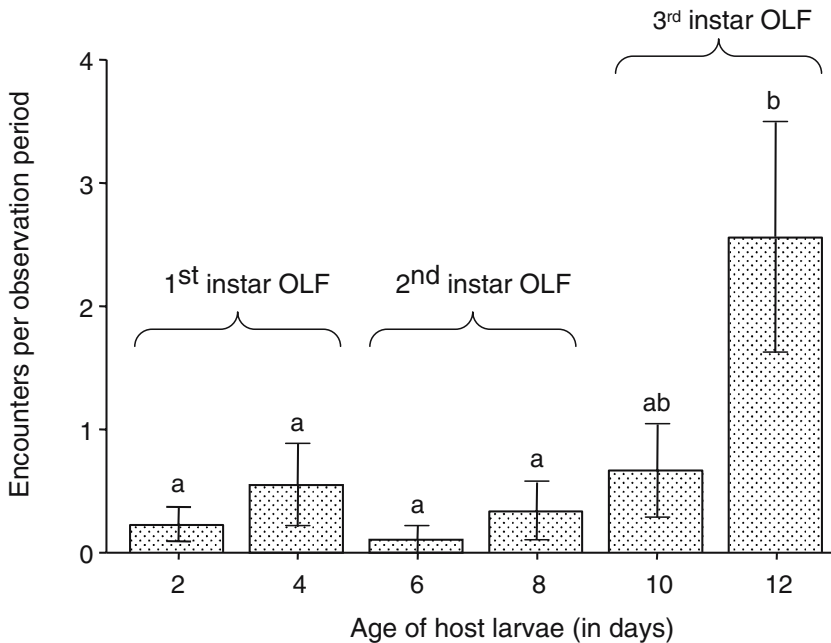


Figure 1. Host-stage preference for *B. celer*, measured as the sum of contact and probing encounters per replicate, was significantly affected by fly (OLF) age ($F=4.141$, $df=5$, 48 , $P=0.003$). Mature (12-day old) third-instar fly larvae were favored over first and second instars (Tukey's pairwise comparisons, $P<0.05$).

successfully reared from the exposed olives ($n=7$), all came from the third-instar olive fly (age class 10 and 12 days), and all but one came from the oldest hosts. Although this result is significant across all age classes (Kruskal–Wallis, ($\chi^2=13.126$, $df=5$, $P=0.002$), there was no significant pairwise separation of individual age class treatments (Mann–Whitney, $P>0.0033$). Host-stage preference has not been reported for *B. celer*, but these results agree with the observation that its larvae are found on third-instar olive flies (Neuenschwander, 1982). Our dissections of olives with parasitized hosts confirmed that *B. celer* is an idiobiont, the larvae feeding on the same host stage upon which the egg was laid. It develops as a solitary ectoparasite that completes its development on a single, immobilized host.

The ectoparasitic-idiobiont habit is typical of the subfamily Braconinae (Quicke, 1997). It distinguishes *B. celer* from other parasitoids of the olive fly and other Tephritidae, which are typically endoparasitic koinobionts (Wharton, 1989; Wharton et al., 2000). Under identical experimental conditions, the opiine braconids we have also studied, *Diachasmimorpha* and *Psytalia* species, deposited eggs in

younger olive fly larvae, typically second and young third instars, and completed their development in the flies' puparia (Sime et al., 2006; and unpublished data). These results suggest that interspecific competition among parasitoids will be a concern if *B. celer* is to be included in a field-release program. As an external parasitoid of nearly-mature larvae, *B. celer* may have a competitive advantage over species that develop internally and oviposit into younger hosts. It will be important to determine whether *B. celer* will attack a host previously parasitized by an endoparasitoid, and the outcome when the same host is exposed to both *B. celer* and another species (Denoth et al., 2002; Wang et al., 2003).

Adult longevity as a function of provisioning

Maximum adult female longevity occurred in the three treatments that included honey (Figure 2), with the wasps living 19–51 days. Of these treatments, longevity was shortest when the parasitoids were provided hosts and longest when no hosts or olives were provided. The longevity of *B. celer* females when provided honey and water was

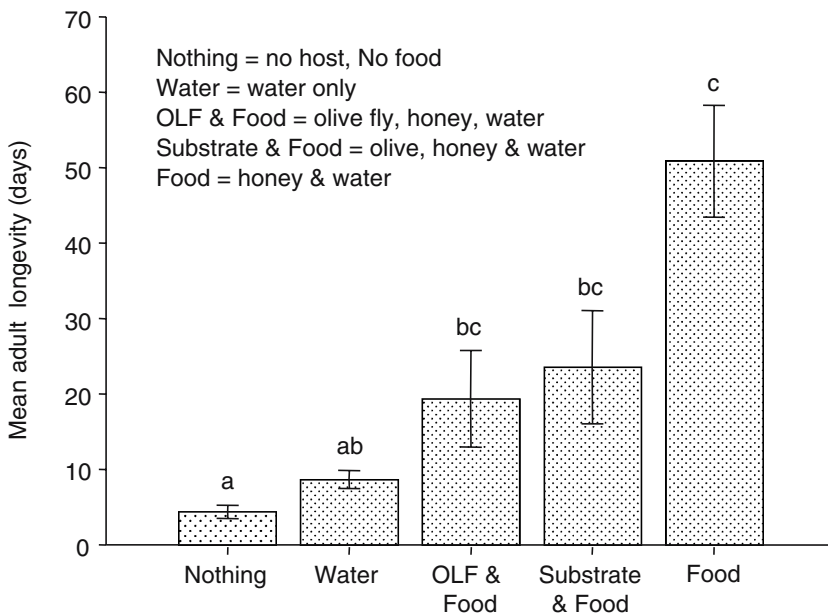


Figure 2. Adult female longevity was significantly affected by provisioning ($\chi^2 = 27.04$, $df = 4$, $P < 0.001$). Adults in the honey treatments lived significantly longer on average than those with no food provision ($P < 0.005$).

longer than that reported for other *Bracon* species with similar provisions (e.g. Jackson and Butler, 1984: 18.9 days; Youm and Gilstrap, 1993: 24.7 days). Adult longevity was significantly lower in the treatment without food or water.

The results of our fecundity study must be considered preliminary, for only six females produced viable offspring in the course of the experiment. Difficulties with maintaining the colony late in the season precluded further replication. The mean number of offspring produced was low (9.7 ± 7.2 adults reared per female tested) compared to other braconines, although comparisons of *B. celer* to other braconines may not be appropriate because the hosts and life-histories differ. Typical lifetime fecundities reported for *Bracon* species are in the range of 100–400 progeny (Engroff and Watson, 1975; Barfield et al., 1977; Jackson and Butler, 1984; Ode et al., 1996). Other braconids parasitizing the olive fly produce 20–30 offspring in captivity (Sime et al., 2006), which may be a more realistic comparison. We suspect that the low progeny production we observed is a consequence of the experimental design and therefore can be improved. Three possible explanations for the low observed fecundity are (1) the number of hosts offered was insufficient, and they were killed by the female wasp due to repeated probing or injection of venom; (2) insufficient hosts led to superparasitism; or (3) the host larvae or the olives themselves were unsuitable for oviposition and development. The first two explanations may have some merit, as there is evidence that exposure to *B. celer* females causes significant mortality for both the fly and *B. celer* larvae, perhaps through excessive probing, or superparasitism (H. Nadel, unpublished data). For rearing purposes, both problems can be minimized by increasing the ratio of hosts to wasps. The third possibility can be ruled out because *B. celer* was simultaneously being successfully reared at much higher numbers using olive fly hosts and olives that were similar to those used in the fecundity study. The oviposition chamber used in colony maintenance was much larger than that used in the fecundity study, however, which suggests a fourth possible explanation: the smaller space may have disrupted host location or oviposition behaviors. The oviposition behavior of many braconid species is stimulated by specific combinations of odors derived from the plant, the plant–host complex, and the host itself (Henson et al., 1977; Strand et al., 1989; Guerra et al., 1994; Darwish et al., 2003; Faccoli and Henry, 2003), and in a smaller arena such cues may have been present in excess or in inappropriate ratios.

Pre-imaginal development time

The preimaginal development time (oviposition to adult eclosion) for male *B. celer* was 33.8 ± 0.2 days at a constant temperature of 22 ± 2 °C; for females it was 35.5 ± 0.8 days (which does not differ significantly from the male development time). These values are nearly three times as long as those reported for other *Bracon* species reared under similar conditions, including *B. kirkpatricki* (Wilkinson) (Engroff and Watson, 1975), *B. brevicornis* Wesmael (Abbas, 1980), *B. greeni* Ashmead (Jackson and Butler, 1984), and *B. hebetor* Say (Sekhon and Varma, 1983; Jackson and Butler, 1984), although *B. hebetor* required 15–17 days to develop when reared on an artificial diet (Magro and Parra, 2004). These and other related braconine species for which data are available may not be well suited for comparison, however, as they are usually gregarious and attack mainly the larvae of Coleoptera and Lepidoptera. Less is known about the biology of parasitoids reared on the olive fly, in part because of difficulties in maintaining parasitoid colonies. We found that the development times for *Diachasmimorpha kraussii* (Fullaway) and *D. longicaudata* (Ashmead), reared on olive fly under the same conditions in the same facility, ranged from 17–20 days (Sime et al., 2006). Being koinobionts, however, these and other braconid parasitoids of tephritid larvae are not very appropriate for comparison of development rate.

Temperature and development times are particularly important for effective insectary rearing of *B. celer* because it is most successfully reared in mature but not very ripe fruit, i.e. green fruit with the endocarp softening throughout. This situation allows only a relatively narrow window for optimum development in culture. Once picked, the olives continue to ripen, and if already purple or black at the time of oviposition they are often rotten and oily before the parasitoid finishes its development. As an ectoparasitoid that completes its entire development within the fruit, *B. celer* appears to be more sensitive to the condition of the fruit than the endoparasitoids, which are carried away from the fruit inside host larvae and pupate elsewhere. In particular, *B. celer* may suffer increased mortality in dried or rotten fruit. Very few *B. celer* were successfully reared from the black, ripe olives that were used towards the end of the growing season.

This problem is mainly a concern for the insectary, where picked fruit must be used, and unlikely to affect populations established in the field. EBCL surveys of olive fly populations in Namibia and

South Africa found that *B. celer* was one of the most commonly reared parasitoids from infested wild olives collected from late March to mid May. The development of wild olives varied widely during this time and ranged from all green fruit to all ripe fruit found on different trees within the same general area, and sometimes this range occurred on different portions of the same tree. Separate collections were made of green and ripe olives whenever possible, and *B. celer* emerged from both groups in comparable numbers (K. Hoelmer, unpublished data/in preparation). Surveys of commercial olives, which tend to be larger and oilier than wild olives, in South Africa indicate a similar pattern, with *B. celer* abundant through June on ripening olives and becoming rarer only at the very end of the growing season (V. Walton, unpublished data).

Insectary rearing and parasitoid release

Our studies provide information on the host-stage preference and adult longevity that may be useful in insectary production. Problems encountered in the fecundity study and during colony maintenance provided useful information as well. The low number of offspring produced in the fecundity study suggests that sufficient hosts must be provided for optimal reproduction, and that containers must be large enough to avoid disruption of oviposition behavior. In routine colony maintenance, *B. celer* was at first easy to rear on infested olives. From the initial eight females, the colony was maintained at high levels for three months (July through September), producing a total of 1011 adult *B. celer* with a sex ratio (male:female) of approximately 1.2:1. Production was intentionally slowed in September because the *B. celer* population began to exceed available resources. In November, most of the olives still available in the field were ripe. At this point we were still able to maintain the fly colony but the parasitoid colony was lost.

These results suggest that fruit quality influences oviposition decisions by *B. celer*, either through volatile production or through tactile cues, and has an effect on larval and pupal survival as well. One possible way to overcome periods of poor olive quality is to use artificial diets. Although effective artificial diets are available for olive fly (Tzanakakis, 1989), we have not been able to induce *B. celer* females to oviposit on olive flies reared and presented in artificial diet. Further tests should be conducted to determine whether this problem can be overcome by including extracts of green olives in the diet, to

provide the plant volatiles that may be necessary to stimulate oviposition (see Eitam et al., 2003). Another option may be the development of artificial diets for *B. celer* itself, without hosts, as has been accomplished for other *Bracon* species that parasitize moth (Magro and Parra, 2004) and beetle (Guerra et al., 1993) larvae.

There are two central concerns that must be resolved before *B. celer* can be released in California. As this species is probably not a specialist on olive fly, it is necessary to determine the level of risk its release might pose to other tephritids present in California, which include a variety of native species and introduced beneficials (Duan et al., 1996; Messing, 2000; Hoddle, 2004). Non-target studies are currently underway. The second concern is whether *B. celer* has a competitive advantage over other olive fly parasitoids. Although in some systems fruit-fly parasitoids partition resources or habitats and complement each other, resulting in lower pest densities overall (O'Neil and Cate, 1985; Wang and Messing, 2003; Wang et al., 2003), competition for the same resources can present problems for parasitoid establishment and effectiveness in the field (Denoth et al., 2002). If *B. celer* attacks olive fly for only a limited period of the season and reduces densities of other, possibly more effective parasitoid species during that period, then parasitism may not reach its potential over the course of the season. For this reason, quarantine evaluation must include studies of interspecific competition and comparative parasitoid effectiveness so that appropriate combinations of parasitoid species may be selected for release.

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References

- Abbas, M.S.T., 1980. *Bracon brevicornis* Wesm., a larval parasite of the pink bollworm, *Pectinophora gossypiella* Saunders. *Agric. Res. Rev.* 58: 233–238.
- Annecke, D.P. and V.C. Moran, 1982. *Insects and Mites of Cultivated Plants in South Africa*. Butterworths, Durban/Pretoria.
- Barfield, C.S., D.G. Bottrell and J.W. Smith Jr., 1977. Influence of temperature on oviposition and adult longevity of *Bracon mellitor* reared on boll weevils. *Environ. Entomol.* 6: 133–137.
- Clausen, C.E., 1978. Introduced parasites and predators of arthropod pests and weeds: a world review. *Agriculture handbook No. 480*. United States Department of Agriculture, Washington, DC.
- Daane, K.M., R.E. Rice, W.W. Barnett, F.G. Zalom and M.W. Johnson, 2005. Arthropod pests. In: G. Sibbett and L. Ferguson (eds), *Olive Production Manual*. Univ. Calif. Div. Agric. Natr. Resources, Berkeley, California. pp. 105–114.
- Darwish, E., M. El-Shazly and H. El-Sherif, 2003. The choice of probing sites by *Bracon hebetor* Say (Hymenoptera: Braconidae) foraging for *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *J. Stored Prod. Res.* 39: 265–276.
- Denoth, M., L. Frid and J. Myers, 2002. Multiple agents in biological control: improving the odds? *Biol. Control* 24: 20–30.
- Duan, J.J., M.F. Purcell and R.H. Messing, 1996. Parasitoids of non-target tephritid flies in Hawaii: implications for biological control of fruit fly pests. *Entomophaga* 41: 245–256.
- Eitam, A., T. Holler, J. Sivinski and M. Aluja, 2003. Use of host fruit chemical cues for laboratory rearing of *Doryctobracon areolatus* (Hymenoptera: Braconidae), a parasitoid of *Anastrepha* spp. (Diptera: Tephritidae). *Fla. Entomol.* 86: 211–216.
- Engroff, B.W. and T.F. Watson, 1975. Influence of temperature on adult biology and population growth of *Bracon kirkpatricki*. *Ann. Entomol. Soc. Am.* 68: 1121–1125.
- Faccoli, M. and C.J. Henry, 2003. Host location by chemical stimuli in *Bracon hylobii* (Ratzeburg) (Hymenoptera: Braconidae), a larval parasitoid of *Hylobius abietis* (L.) (Coleoptera: Curculionidae). *Ann. Soc. Entomol. France* 39: 247–256.
- Guerra, A.A., S. Martinez and H.S. Delrio, 1994. Natural and synthetic oviposition stimulants for *Catolaccus grandis* (Burks) females. *J. Chem. Ecol.* 20: 1583–1594.
- Guerra, A.A., K.M. Robacker and S. Martinez, 1993. In vitro rearing of *Bracon mellitor* and *Catolaccus grandis* with artificial diets devoid of insect components. *Entomol. Exp. Appl.* 68: 303–307.
- Henson, R.D., S.B. Vinson and C.S. Barfield, 1977. Ovipositional behavior of *Bracon mellitor* Say, a parasitoid of boll weevil (*Anthonomus grandis* Boheman) III. isolation and identification of natural releasers of ovipositor probing. *J. Chem. Ecol.* 3: 151–158.
- Hoddle, M.S., 2004. Restoring balance: using exotic species to control invasive exotic species. *Conserv. Biol.* 18: 38–49.
- Hoelmer, K., A. Kirk, R. Wharton and C. Pickett, 2004. Foreign exploration for parasitoids of the olive fruit fly, *Bactrocera oleae*. In: D. Woods (ed.), *Biological Control Program Annual Summary, 2003*. California Dept. of Food & Agriculture, Sacramento. pp. 12–14.
- Jackson, C.G. and G.D. Butler, 1984. Development time of three species of *Bracon* (Hymenoptera, Braconidae) on the pink bollworm (Lepidoptera: Gelechiidae) in relation to temperature. *Ann. Entomol. Soc. Am.* 77: 539–542.

- Magro, S.R. and J.R.P. Parra, 2004. Comparison of artificial diets for rearing *Bracon hebetor* Say (Hymenoptera: Braconidae). *Biol. Control* 29: 341–347.
- Messing, R.H., 2000. The impact of non-target concerns on the practice of biological control. In: P. Follett and J.J. Duan (eds), *Non-target Effects of Biological Control*. Kluwer Academic Publishers, Norwell, Massachusetts. pp. 45–55.
- Narayanan, E.S. and S.S. Chawla, 1962. Parasites of fruit fly pests of the world. *Beitr. Entomol.* 12: 437–476.
- Neuenschwander, P., 1982. Searching parasitoids of *Dacus oleae* in South Africa. *J. Appl. Entomol.* 94: 509–522.
- Ode, P.J., M.F. Antolin and M.R. Strand, 1996. Sex allocation and sexual asymmetries in intra-brood competition in the parasitic wasp *Bracon hebetor*. *J. Anim. Ecol.* 65: 690–700.
- O'Neil, R.J. and J.R. Cate, 1985. Competition between *Bracon mellitor* (Hym: Braconidae) and *Catolaccus grandis* (Hym: Pteromalidae) for their host *Anthonomus grandis* (Col: Curculionidae). *Entomophaga* 30: 375–384.
- Quicke, D.L.J., 1997. Subfamily Braconinae. In: R.A. Wharton, P.M. Marsh and M.J. Sharkey (eds), *Manual of the New World Genera of the Family Braconidae (Hymenoptera)*. International Society of Hymenopterists, Washington, DC. pp. 149–174.
- Rice, R.E., P. Phillips, J. Stewart-Leslie and G. Sibbett, 2003. Olive fruit fly populations measured in central and southern California. *Calif. Agric.* 57: 122–127.
- Sekhon, B.S. and G.C. Varma, 1983. Parasitoids of *Pectinophora gossypiella* (Lep, Gelechiidae) and *Earias* spp. (Lep., Noctuidae) in the Punjab. *Entomophaga* 28: 45–53.
- Shaw, M.R., 1994. Parasitoid host ranges. In: B.A. Hawkins and W. Sheehan (eds), *Parasitoid Community Ecology*. Oxford University Press, Oxford. pp. 111–144.
- Silvestri, F., 1914. Report on an expedition to Africa in search of the natural enemies of fruit flies (Trupaneidae) with descriptions, observations and biological notes. *Terr. Hawaii Board Agric. For. Div. Entomol. Bull.* 3: 1–146.
- Sime, K.R., K.M. Daane, H. Nadel, C.S. Funk, R.H. Messing, J.W. Andrews Jr., M.W. Johnson and C.H. Pickett, 2006. *Diachasmimorpha longicaudata* and *D. kraussii* (Hymenoptera: Braconidae), potential parasitoids of the olive fruit fly. *Biocontrol Sci. Tech.* 16: 169–179.
- SPSS, 2000. SYSTAT 10.0. SPSS, Chicago, Illinois, USA.
- Strand, M.R., H.J. Williams, S.B. Vinson and A. Mudd, 1989. Kairomonal activities of 2-acylcyclohexane-1,3 diones produced by *Ephestia kuehniella* Zeller in eliciting searching behavior by the parasitoid *Bracon hebetor* (Say). *J. Chem. Ecol.* 15: 1491–1500.
- Tzanakakis, M., 1989. Small scale rearing: *Dacus oleae*. In: A. Robinson and G. Hooper (eds), *Fruit Flies: Their Biology, Natural Enemies and Control*. Elsevier, Amsterdam. pp. 105–118.
- Tzanakakis, M., 2003. Seasonal development and dormancy of insects and mites feeding on olive: a review. *Neth. J. Zool.* 52: 87–224.
- Wang, X.G. and R.H. Messing, 2003. Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. *Biol. Control* 27: 251–259.
- Wang, X.G., R.H. Messing and R.C. Bautista, 2003. Competitive superiority of early acting species: a case study of opiine fruit fly parasitoids. *Biocontrol Sci. Tech.* 13: 391–402.

- Wharton, R.A., 1989. Classical biological control of fruit-infesting Tephritidae. In: A. Robinson and G. Hooper (eds), *Fruit Flies: Their Biology, Natural Enemies and Control*. Vol. 3B. Elsevier, Amsterdam. pp. 303–313.
- Wharton, R.A., M.K. Trostle, R.H. Messing, R.S. Copeland, S.W. Kimani-Njogu, S. Lux, W.A. Overholt, S. Mohamed and J. Sivinski, 2000. Parasitoids of medfly, *Ceratitidis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. *Bull. Entomol. Res.* 90: 517–526.
- White, I. and M. Elson-Harris, 1992. *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International, Oxon, U.K.
- Youn, O. and F.E. Gilstrap, 1993. Life-fertility tables of *Bracon hebetor* Say (Hymenoptera: Braconidae) reared on *Heliocheilus albipunctella* De-Joannis (Lepidoptera: Noctuidae). *Ins. Sci. Appl.* 14: 455–459.